Research note

Effect of nitrogen supply on expression of some genes controlling storage proteins and carbohydrate synthesis in cultured maize kernels*

Douglas C. Doehlert[1], Edwin R. Duke[2] & Leslie J. Smith[3]
[1] *USDA/ARS Wheat Quality Laboratory, Harris Hall, North Dakota State University, Fargo, ND 58105, USA;*
[2] *University of Florida, Ft. Lauderdale Research and Education Center, Ft. Lauderdale, FL 33314, USA;*
[3] *USDA/ARS National Center for Agricultural Utilization Research, 1815 N. University Street, Peoria, Illinois 61604 USA*

Abstract

Maize kernels (*Zea mays* L., inbred W64A) were cultured *in vitro* with a 200 mM sucrose concentration and either 0, 3.6, 7.1, 14.3, or 36.5 mM N, supplied as amino acids, in the culture medium. Increased N supply up to 14.3 mM N resulted in increased kernel dry weight and starch content. Increasing N supply increased storage protein 3-fold, which was reflected by 7-fold enhancement of α-zein message abundance. β-Zein message abundance was not affected by N nutrition. Messages for the regulatory gene, *Opaque-2*, were doubled by the increased N supply. Messages for the *Shrunken, Waxy,* and *Aldolase* genes increased about 2-fold with increased N supply. Activities of sucrose synthase (encoded by *Shrunken*), and aldolase enzymes exhibited increases with N supply corresponding with that of their transcript abundance. Expression of the *Shrunken-2* and *Brittle-2* genes were not affected by N supply. It is likely that the increased expression of genes involved in carbohydrate metabolism is responsible for the increased capacity of a kernel to utilize available sucrose at higher levels of N nutrition.

Abbreviations: DPP – days postpollination

Agricultural production of maize (*Zea mays* L.) generally requires heavy application of nitrogen fertilizer for optimal yields (Balko and Russell, 1980). Although most of the increase in grain yield with high nitrogen nutrition represents increases in ear sizes and number of kernels produced per plant, maize plants grown with increased nitrogen supplies produce larger kernels with increased protein concentrations (Tsai et al., 1978). The development of an *in vitro* culture method for developing maize kernels (Gengenbach, 1977) has allowed the examination of the effects of N nutrition on the developing kernel itself, isolated from the mother plant. Singletary et al., (1990) demonstrated that activities of several enzymes key to sucrose and amino acid

metabolism were increased substantially by increased nitrogen supply. It was concluded that the increased nitrogen supply allowed greater enzyme synthesis that increased the capacity of the developing kernels to utilize more available carbohydrate.

The most plausible explanation for the observed changes in kernel metabolic activities with nitrogen supply is that the gene expression in the kernel was affected. In this study, we test the hypothesis that nitrogen supply affects maize kernel development by affecting gene expression.

Maize plants (*Zea mays* L. inbred W64A) were grown in a growth room illuminated 15 h d^{-1} with a mixture of high pressure sodium and metal halide lamps supplying 800 mm photons m^{-2}s^{-1} at a height of 2 m. Temperatures were maintained at 26°C days, 18°C nights. Plants were grown in 12 L pots in Redi-

earth Peat-lite soilless medium (W.R. Grace, Cambridge, MA) supplemented with Osmocote 14-14-14 and Micromax micronutrients (Sierra, Militas, CA). Individual ears were bagged as they appeared and either self- or sib-pollinated at silk emergence. Five days postpollination (DPP), kernels were cultured *in vitro* essentially as described by Gengenbach (1977). Explants were placed into 100 x 25 mm Petri plates on an agar-based medium described by Singletary and Below (1989), except nitrogen was supplied at five different concentrations (0, 3.6, 7.1, 14.3, 36.5 mM) as amino acids from casein hydrolysate. Cultured kernels (five per Petri plate) were incubated in darkness at 25°C. Plates were harvested at 20 DPP (15 days after culturing) and at maturity (45 DPP). Two kernels were taken from each plate harvested at 20 DPP and frozen at -80°C for RNA extraction and analysis. The remaining three kernels were frozen on dry ice, lyophilized, ground to a powder and stored at -80°C until used for enzyme extractions. Four replications were made for each N concentration for both mature and immature kernels.

Enzyme extractions and assays were performed as described by Doehlert et al. (1988). Starch, total protein and zein determinations were as described earlier (Singletary et al., 1990).

For each RNA extraction, two frozen kernels (about 0.5 g fresh weight) were ground to a fine powder in liquid N_2 using a mortar and pestle. RNA was extracted and purified and dot-blots of total RNA performed as described by Doehlert and Kuo (1994).A cDNA clone of *Shrunken-1* (pZMc1106) and of *Aldolase* (pZMc1154) were obtained from Dr. P. Kelley (University of Nebraska, Lincoln, NE). A cDNA clone of the maize *Waxy* gene was obtained from Dr. S. Wessler (University of Georgia, Athens, GA). A cDNA clone of *Shrunken-2* (pES6-66) and of *Brittle-2* (pES6-75) were obtained from Dr. C. Barton (ESCAgenetics, San Carlos, CA). A genomic clone of α-*zein*, (pZ19ab1) and a genomic clone of β-*zein* (pZG15.1) were supplied by Dr. B.A. Larkins, (University of Arizona, Tuscon, AZ). A cDNA clone of *Opaque-2* was supplied by Dr. R.J. Schmidt (University of California, San Diego, CA).

Analysis of variance and least significant differences were calculated with the Statistix computer package (Analytical Software, Tallahassee, FL, USA)

Results indicated that increasing N supply up to 14.5 mM N increased mature kernel dry weight and starch contents, but both starch and dry weight were lower in mature kernels grown at 34.5 mM N (Fig-
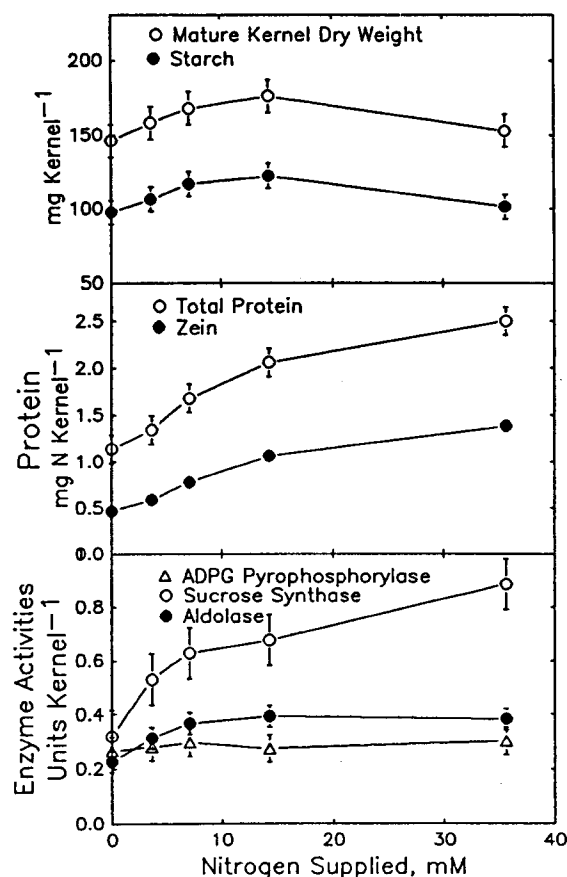

Figure 1. Effect of nitrogen nutrition on mature kernel dry weight, starch content, total protein, zein content, and enzyme activities. Data points are means, $n = 4$. Error bars are least significant differences. Values in the same sets whose error bars do not overlap are significantly different ($\alpha=0.05$).

ure 1). Total nitrogen content per mature kernel doubled and zein nitrogen nearly tripled with increased nitrogen supply (Figure 1). The relative proportion of zein protein to total protein also increased slightly with increased nitrogen supply. Sucrose synthase activity, extracted from kernels harvested 20 DPP was stimulated nearly 3 fold by the increased nitrogen supply (Figure 1). Aldolase activity increased about 2-fold, whereas there was no effect upon activity of ADP-Glc pyrophosphorylase by increased nitrogen supply (Figure 1).

The relative abundance of transcript for α-*Zein* in kernels harvested at 20 DPP increased by over 7-fold with the increased nitrogen supply (Figure 2). This was the largest increase in relative transcript abundance of any of the transcripts measured in this study. In con-
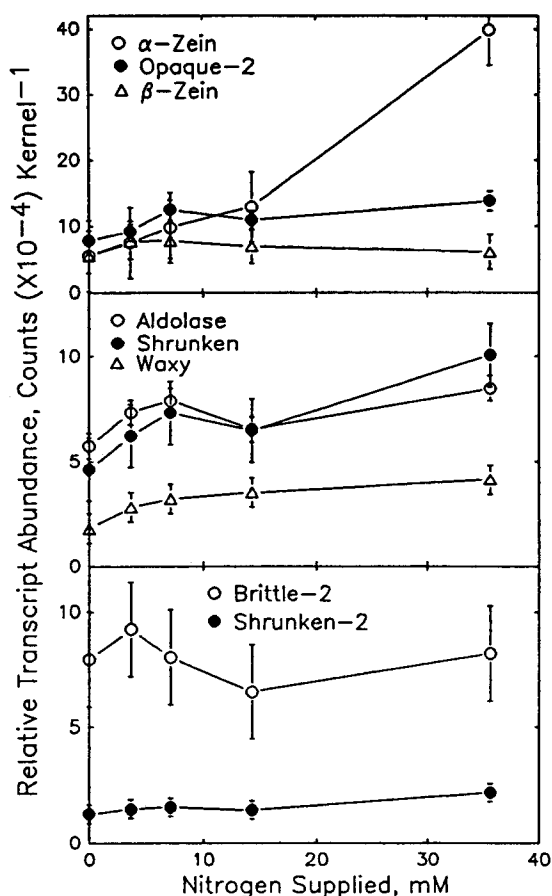
Figure 2. Effect of nitrogen nutrition on relative abundance of transcript for *α-Zein*, *β-Zein*, *Opaque-2*, *Aldolase*, *Shrunken-1*, *Waxy*, *Brittle-2*, and *Shrunken-2* in developing maize kernels grown *in vitro*, harvested 20 DPP. Data points are means, *n* = 4. Error bars are least significant differences. Values in the same sets whose error bars do not overlap are significantly different ($\alpha=0.05$).

trast, the relative abundance of *β-Zein* transcript in kernels harvested at 20 DPP was not affected by nitrogen supply. The relative abundance of *Opaque-2* transcript doubled with increasing nitrogen supply (Figure 2). Relative abundance of transcripts for *Aldolase*, *Shrunken-1* and *Waxy* genes was about doubled by the increasing nitrogen supply (Figure 2). These transcripts encode cytosolic aldolase, sucrose synthase and starch-granule bound starch synthase, respectively. Nitrogen supply did not affect transcript abundance of *Brittle-2* or *Shrunken-2* (Figure 2) which encode for the two subunits of ADP-Glc pyrophosphorylase.

These results indicate that a major effect of increased nitrogen nutrition on maize kernel development is on gene expression. Whereas changes in starch accumulation can be attributed to increased activities of enzymes of carbohydrate metabolism (Figure 1), the increased enzyme activities and increased storage protein accumulation can be attributed to increased transcript abundance of genes encoding for these enzymes and storage proteins (Figure 2). Balconi et al. (1993) have also reported that increased N supply increased levels of α-zein transcripts. It is interesting to observe that increased nitrogen nutrition stimulated *α-Zein* transcript levels so much whereas *β-Zein* transcript levels were unaffected (Figure 2). This observation is consistent with an earlier study (Singletary et al., 1990) that indicated that increased nitrogen greatly stimulated α-zein peptide abundance, but had no effect on the accumulation of *β*-zein peptide. Increasing nitrogen supply about doubled the transcript abundance of the regulatory gene *Opaque-2*. Because of the evidence indicating that the gene product of the *Opaque-2* gene activates transcription of *α-Zein* genes (Schmidt et al., 1990), it is possible that the increased transcript levels of the *Opaque-2* gene led to the increased *α-Zein* transcript and peptide abundance.

The increased transcript levels for *Aldolase* and *Shrunken-1* (Figure 2) correspond well with observed changes in activities of aldolase and sucrose synthase (Figure 1). An earlier study concluded that the increase in nitrogen nutrition of developing maize kernels appeared to increase the capacity of the kernels to utilize available sucrose, largely through increasing activities of enzymes of sucrose metabolism (Singletary et al., 1990). In this study, we conclude that the increase in enzyme activities (Figure 1) are likely to be the result of increased transcript abundance (Figure 2). The mechanism by which nitrogen nutrition affects transcript abundance was not addressed in this study. But, because nitrogen supply affects some genes more than others and some not at all, it appears to be a fairly specific control mechanism and it would appear that those genes affected have adaptive significance. Increased α-zein transcript would allow for increased storage protein accumulation, and increased *Shrunken-1*, *Waxy* and *Aldolase* transcripts would allow for increased enzyme activity which would enhance the metabolic capacity of the maize kernel to convert sucrose into storage products.

References

Balconi C, Rizzi E, Motto M, Salamini F & Thompson R (1992) The accumulation of zein polypeptides and zein mRNA in cultured

endosperms is modulated by nitrogen supply. Plant J. 3: 325–334

Balko LG & Russell WA (1980) Response of maize inbred lines to N fertilizer. Agron. J. 72: 723–728

Doehlert DC, & Kuo TM (1994) Gene expression in developing kernels of some endosperm mutants of maize. Plant Cell Physiol. 35: 411–418.

Doehlert DC, Kuo TM & Felker FC (1988) Enzymes of sucrose and hexose metabolism in developing kernels of two inbreds of maize. Plant Physiol. 86: 1013–1019

Gegenbach BG (1977) Development of maize caryopses resulting from in vitro pollination. Planta 134: 91–93

Schmidt RJ, Burr FA, Aukerman MJ & Burr B (1990) Maize regulatory gene Opaque-2 encodes a protein with a "leucine-zipper" motif that binds to zein DNA. Proc. Natl. Acad. Sci. USA 87: 46–50

Singletary GW & Below FE (1989) Growth and composition of maize kernels cultured in vitro with varying supplies of carbon and nitrogen. Plant Physiol. 89: 341–346

Singletary GW, Doehlert DC, Wilson CM, Muhitch MJ & Below FE (1990) Response of enzymes and storage proteins of maize endosperm to nitrogen supply. Plant Physiol. 94: 858–864

Tsai CY, Huber DM & Warren HL (1978) Relationship of the kernel sink of N to maize productivity. Crop Sci.18: 399–404